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INTERLEUKIN-8 T-251A POLYMORPHISM WAS ASSOCIATED WITH POSITIVE ANTI-p53 ANTIBODIES IN UZBEKISTAN POPULATION

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ABSTRACT

Interleukin (IL)-8, a proinflammatory chemokine, has been reported to have angiogenic activity and to be responsible for tumor-associated angiogenesis in several cancers. The polymorphism *IL-8 T-251A* (rs4073) is known to be associated with the expression of IL-8 protein and is related to several cancers. A serum anti-p53 antibody, a new tumor marker, increases in accordance with the mutation of tumor suppressor gene *p53*. Previous studies have reported the association between IL-8 and *p53* mutation in cancer cells or tissues. Therefore, we hypothesized that *IL-8* polymorphism might be associated with serum anti-p53 antibody levels. Study subjects were 197 participants (103 males and 94 females, aged 15 to 56 years) who were enrolled in a case-control study on peptic ulcer disease from January to March 2007 in the Uzbekistan Republic. Serum anti-p53 antibody, CEA, and CA19-9 levels were measured, and *IL-8 T-251A* was genotyped. The *A* allele frequency in control subjects was 0.48, which is close to the previous reports for Caucasian populations. The proportion of subjects with positive anti-p53 antibodies (higher than 1.3 U/mL) was greater for *AA* genotype carriers compared to *T* allele carriers (17% for *AA*, and 6% for *TA+TT*; OR 3.4, p = 0.025 after adjusting for age, sex, comorbidity and ethnicity). Such a difference was not observed for either CEA or CA19-9. We demonstrated that the *IL-8 -251 AA* genotype was associated with higher anti-p53 antibodies than those of the reference range. Further studies are warranted to clarify whether those with this genotype carrier are susceptible to malignant diseases.

Key Words: Interleukin-8, Anti-p53 antibody, Genetic polymorphism, Uzbekistan

INTRODUCTION

Interleukin (IL)-8, a proinflammatory chemokine, has been reported to exhibit angiogenic activity and to be responsible for tumor-associated angiogenesis in several cancers.[1,2] The polymorphism *IL-8 T-251A* (rs4073) is located at the promoter region, and it's *A* allele carrier is known to show an elevated expression of IL-8 protein.[3,4] This polymorphism was shown to be associated with several malignancies including gastric,[3,5,6] nasopharyngeal,[7] and colorectal cancers.[8]

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The mutation of the tumor suppressor gene *p53* is reported to be one of the most common genetic changes found in malignant tumors.[9] A serum anti-p53 antibody, which is elevated in accordance with the *p53* mutation,[10] is used clinically to increase the diagnostic sensitivity of conventional tumor markers.

Previous studies have demonstrated the association between IL-8 and *p53* mutation or p53 expression in cancer cells/tissues.[11-14] Therefore, we hypothesized that *IL-8* polymorphism might be associated with higher serum anti-p53 antibody levels.

SUBJECTS AND METHODS

Study subjects

Study subjects were derived from a case-control study on peptic ulcer diseases (PUD) in the Uzbekistan Republic as described in our previous study.[15] In brief, there were 197 participants (103 males and 94 females, aged 15 to 86 years) enrolled from January to March 2007 at the Republic Research Center of Emergency Medicine (RRCEM) in Tashkent; cases were comprised of a consecutive 95 patients with PUD (85 duodenal ulcer and 10 gastric ulcer); controls were 102 arbitrarily invited volunteers including 42 patients with miscellaneous diseases (17 acute cholecystitis, 14 acute appendicitis, 4 hernia, 3 pancreatitis, 2 echinococcus infection, 1 nephritis, and 1 pneumonia) as well as 60 students/staffs of the Center (subjects with known malignancies were excluded). Their ethnicities were 161 Uzbeks, 23 Russians, 4 Kazaks, 4 Koreans, and 3 others (1 Tatar, 1 Afghan, and 1 Armenian). Information on age, sex, ethnicity, and smoking/drinking habits were obtained from medical records (patients) and questionnaire/interviews (healthy volunteers). Written informed consent was obtained from all the participants. The study was approved by the Ethics Committee of the Nagoya University School of Medicine (approval number 459).

Biochemical analysis

From each enrolled subject 7.5 ml of blood was drawn. Sera was immediately separated from whole blood and stored at –20°C. Serum anti-p53 antibodies were measured using ELISA (MESACUP anti-p53 test, MBL, Nagoya, Japan). Serum CEA and CA19-9 were also measured using the CLEIA method (SRL, Tokyo, Japan) to check for potential underdiagnosed malignancies. Sera were considered positive for anti-p53 antibodies, CEA, and CA19-9 if their levels were higher than those of the reference ranges (1.3 U/ml, 5.0 ng/ml, and 37.0 U/ml, respectively).

Genotyping

DNA was extracted from 2.5 ml of heparinized blood with a BioRobot M48 Workstation (QIAGEN Group, Tokyo). *IL-8 T-251A* was genotyped using a polymerase chain reaction with confronting two-pair primers (PCR-CTPP).[16] The PCR amplification of *IL-8 T-251A* was conducted using the primers F1 (5'-CAT GAT AGC ATC TGT AAT TAA CTG) and R1 (5'-CAC AAT TTG GTG AAT TAT CAA A) for the *T* allele (a 169-bp fragment), and F2 (5'-GTT ATC TAG AAA TAA AAA AGC ATA CAA) and R2 (5'-CTC ATC TTT TCA TTA TGT CAG AG) for the *A* allele (a 228-bp fragment). The DNA amplified between F1 and R2 resulted in a 349-bp band common to both alleles. Genomic DNA was used in a 25-μl reaction mixture that contained 0.2 mM dNTPs, 12.5 pmol of each primer, 0.5 U polymerase (AmpliTaq Gold; Applied Biosystems, CA, USA), and 2.5 μl 10× PCR Buffer including 15 mM $MgCl_2$. The amplification conditions were an initial 10 min at 95°C, followed by 30 cycles of 1 min each at 95°C, 58°C and 72°C, and then 5 min at 72°C for a final extension. The DNA products were visualized on

2% agarose gels with ethidium bromide staining.

Statistical analysis

The frequency of the polymorphism was tested against the Hardy-Weinberg equilibrium using the χ^2 test in control subjects, since this polymorphism is known to be associated with PUD cases.[6] Differences in the demographic characteristics were analyzed using the one-way analysis of variance (ANOVA) or the χ^2 test. The odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using unconditional multiple logistic regression analysis after adjusting for age, sex, comorbidity (PUD cases or controls), and ethnicity (Uzbeks or others). STATA ver. 9 (StataCorp, TX, USA) was used for these analyses.

RESULTS

All subjects were successfully genotyped except for one PUD case. The *A* allele frequency

Table 1 Background characteristics of study subjects according to the *IL-8 T-251A* genotype

	AA	*TA*	*TT*	*p*-value [a]
	n=47	*n*=82	*n*=67	
Age (years)	36 ± 19	40 ± 17	42 ± 18	0.194
Female	22 (47)	38 (46)	33 (49)	0.744
PUD cases	20 (43)	38 (46)	36 (54)	0.456
Uzbeks	41 (87)	66 (80)	54 (81)	0.726
Smoker	13 (28)	32 (39)	17 (25)	0.251
Drinker	19 (40)	39 (48)	33 (49)	0.615

Variables are presented as means ± SD for age or numbers (percentages) for others.
[a]The one-way analysis of variance (ANOVA) for age or the χ^2 tests for others.

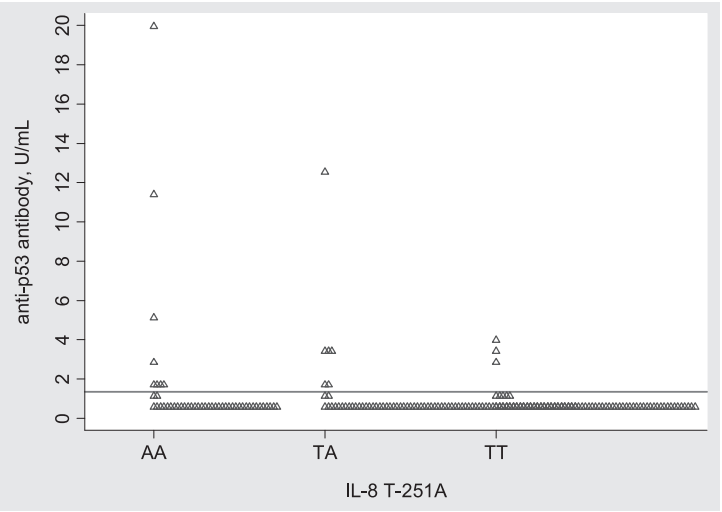

Fig. 1 Distributions of anti-p53 antibodies according to *IL-8 T-251A* genotype

Table 2 Proportion of subjects with positive tumor markers according to *IL-8 T-251A* genotype

	AA	*TA*	*TT*	*TA+TT*	OR[a)]	(95% CI[a)])	*p*-value[a)]
	n=47	*n*=82	*n*=67	*n*=149			
Anti-p53 antibodies, n (%)[c)]	8 (17)	6 (7)	3 (4)	9 (6)	3.4	(1.2–9.0)	0.025
CEA, n (%)[c)]	2 (4)	2 (3)	1 (2)	3 (2)	2.2	(0.3–14.9)	0.595[b)]
CA 19-9, n (%)[c)]	2 (4)	6 (7)	6 (9)	12 (8)	0.6	(0.1–2.8)	0.504[b)]

[a)] *AA* vs. *TA+TT*, adjusted for age, sex, comorbidity and race using unconditional logistic regression models. [b)] Fisher's exact method. [c)] Serum anti-p53 antibody higher than 1.3 U/mL, serum CEA higher than 5.0 ng/mL, and serum CA19-9 higher than 37.0 U/mL.

was 0.48 in controls, which was on the Hardy-Weinberg equilibrium (p = 0.799). The background characteristics were not significantly different between the *IL-8* genotypes (Table 1).

Since the distribution of anti-p53 antibody levels appeared to differ between the *IL-8* genotypes (Fig. 1), the proportion of subjects with positive anti-p53 antibodies was compared between the *AA* genotype and *T* allele carriers. That proportion was higher for *AA* carriers than for *T* allele carriers (17% for *AA*, and 6% for *TA+TT*), and this difference proved to be statistically significant after adjusting for age, sex, comorbidity and ethnicity (OR 3.4, p = 0.025), which differed little between PUD cases and controls (ORs 3.4 and 2.7, respectively). Such differences between the genotypes were not observed for the proportions of positive CEA and CA19-9 (Table 2).

DISCUSSION

The present study demonstrated that the *AA* genotype carriers of the *IL-8* polymorphism had a significantly higher proportion of positive anti-p53 antibodies than the reference range when compared with that of *T* allele carriers. Such a phenomenon has not been observed in other tumor markers, suggesting that it cannot be explained simply by possibly underdiagnosed malignancies.

Our hypothesis about the association between *IL-8* polymorphism and serum anti-p53 antibodies is supported by the following previous studies: the *A* allele carriers produce greater IL-8,[3,4)] it was then found that the neutrophils induced by IL-8 are able to synthesize radicals such as nitric oxide that could cause *p53* mutations,[17,18)] and that those mutations result in higher levels of serum anti-p53 antibodies. Subsequently, Taguchi *et al*. found that *AA* genotype increased the risk of *p53*-mutated subtypes of gastric cancer.[3)] Other biological studies have revealed associations between IL-8 levels and *p53* mutations in cancer cells,[11)] IL-8 levels and the expression of p53 in gastric mucosa,[12)] and expression of *IL-8* mRNA and *p53* mutation[13)] or aberrant p53 expression in lung cancer tissues.[14)] The above findings might explain the association between *IL-8* polymorphism and serum anti-p53 antibody levels.

IL-8 -251 A allele frequency was 0.48 in Uzbekistan controls, a frequency greater than that for Japanese (0.28)[19)] and close to that for Caucasians (0.47).[4)] Such findings are consistent with our previous report that the allele frequency of the Uzbekistan population was somewhat closer to Caucasians than to East Asians.[20)]

In conclusion, we demonstrated that the *IL-8 -251 AA* genotype was associated with positive anti-p53 antibodies rather than with the reference range. Further prospective studies are warranted to clarify whether those with this genotype carrier are susceptible to malignant diseases.

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